Supporting Information for *Chemical Communications*

Evaluation of Aβ fibrillization inhibitory effect by a PEG-peptide conjugate based on an Aβ peptide fragment with intramolecular FRET

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I. Experimental Section

Synthesis

A PEG-peptide conjugate **1** was synthesized manually by solid phase Fmoc (9-fuluorenylmethoxycarbonyl) chemistry using HATU/DIPEA and PEG resin (0.17 mmol g^{-1}; Novabiochem) in NMP. DIPEA was added in 4-fold excess with respect to the amino acid and HATU, and the reaction time was extended to 2 h. Fmoc deprotection was carried out using 20 % piperidine in NMP for two 15 min. The synthesis was followed by UV monitoring of the dibenzofulvene released during the Fmoc deprotection. After the synthesis of 7 residue oligopeptide (KLVFFGW) at the C-terminal, coupled with O-(N-Fmoc-2-aminoethyl)-O'-(2-carboxyethyl)-undecaethyleneglycol (Fmoc-NH-PEG_{12}-COOH) and 6 residue oligopeptide (GKLVFF) at the N-terminal side was synthesized as the same method. After the final deprotection, 4-fold excess of 5-dimethylamino-1-naphthalene sulfonyl chloride (Dansyl-Cl) was added in 50% DMF/NMP for 2 h. The PEGlylated conjugate **1** was cleaved from the resin by treatment with TFA/TIS/H_2O (95/2.5/2.5) for 3 h following by precipitation with cool diethyl ether. The purification was operated at 4.0 ml•min^{-1} using linear A-B gradients in 30 min run time (solvent A, H_2O containing 0.1 % TFA; solvent B, CH_3CN containing 0.1 % TFA) at 25˚C. The eluted **1** was freeze-dried, and stored at -80˚C. Purified **1** was characterized by analytical HPLC (Fig. S1) and MALDI-TOF/MS (Fig. S2): m/z: 2420.57 $[M+H]^+$ (calcd.: m/z: 2419.96).

Analytical HPLC analysis

The purity of **1** was estimated from analytical HPLC reversed-phase high performance liquid chromatography (RP-HPLC; JASCO HPLC system equipment consisting of a JASCO PU-2089i-plus quaternary gradient inert pump, and a JASCO MD-2015plus multiwavelength UV–vis detector) using a ODS column (Inertsil ODS-3, particle size 5 μm, pore size 120 Å, 4.6 mm I.D. x 250 mm, GL Science, Tokyo, Japan) running with H_2O/0.1% TFA (solvent A) and ACN/0.1% TFA (solvent B) gradient (10-25 % B, 20 min) at 1 mL min^{-1}, and was > 95%.

Sample Preparation

Lyophilized **1** was dissolved in 10 mM phosphate buffer (pH 7.0) at 100 μM as a master solution, and stored at -80°C prior to use.

UV Spectroscopy

UV measurements were carried out on a UV/Vis spectrophotometer V-560 (JASCO) at 25°C (Jasco ETC-505T peltier thermostat) using 1.0 cm path-length quartz cell (GL science). Band width: 2.0 nm, scan speed: 100 nm/min.

FRET measurement

Fluorescence spectroscopy was undertaken in order to measure the tryptophan (Trp) emission and FRET spectrum of **1**. 1 μM of **1** in 10 mM phosphate buffer (pH 7.0, total 200 μl) was prepared from 100 μM stock solution, and measured the fluorescence intensity at 505 nm (I_o) using a Jasco spectrometer, model FP-6500 (λ_{ex} = 290 nm, 3 nm bandwidth each) in a 3 mm path-length quartz cell at 25°C. After the measurements, 2.5 μl of each concentrated dye in 10 mM phosphate buffer (pH 7.0) was added into quartz cell, and one minute later measured the fluorescence intensity (I_{add}) again. The intensity ratio (= I_{add}/I_o) was calculated and evaluated as the inhibitory effect.

II. Additional data

Analytical HPLC and TOF/MS spectrum of 1.

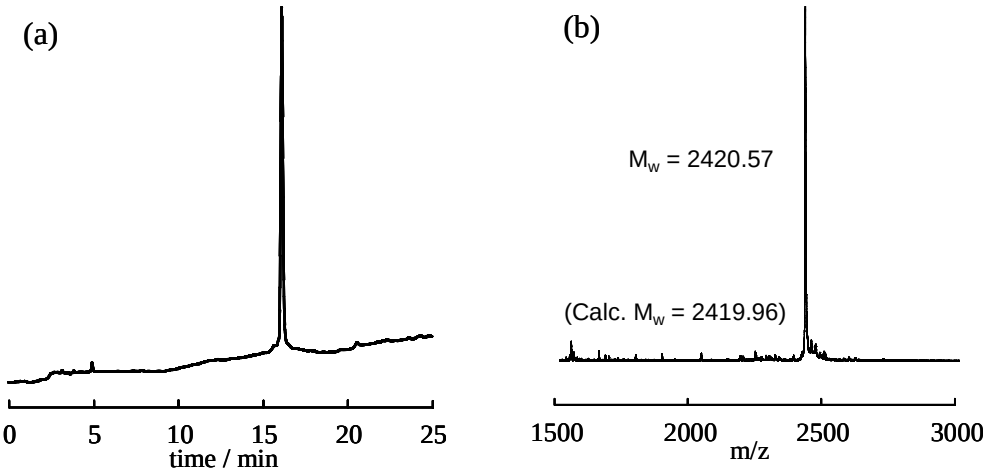


Fig. S1 Analytical HPLC chromatogram (a) and TOF/MS spectrum (b) of **1**.

c.a.c. estimation from UV/Vis spectroscopy

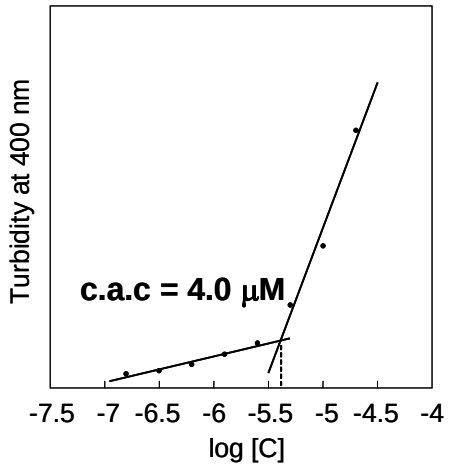


Fig. S2 Concentration dependency on turbidity of **1** in 10 mM phosphate buffer (pH 7.2) at 25°C.

Time course of FRET fluorescence intensity of 1.

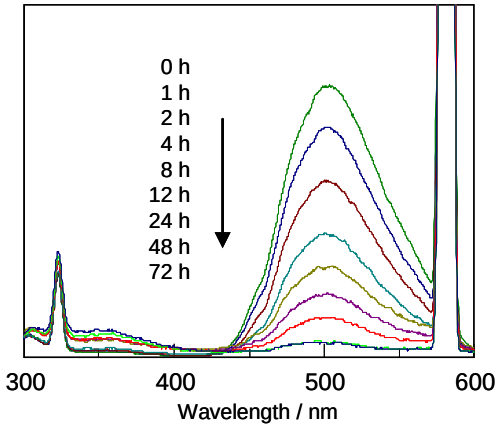


Fig. S3 Time course of FRET spectra of **1** after preparing the master solution at 25°C. [**1**] = 1 μM.

Secondary structure of 1 in each concentration.

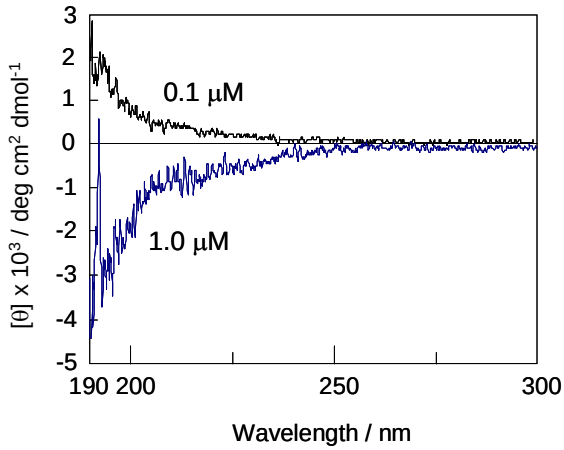


Fig. S4 CD spectra of **1** in 10 mM phosphate buffer at 25°C.

Fluorescence intensity of Trp with denaturants.

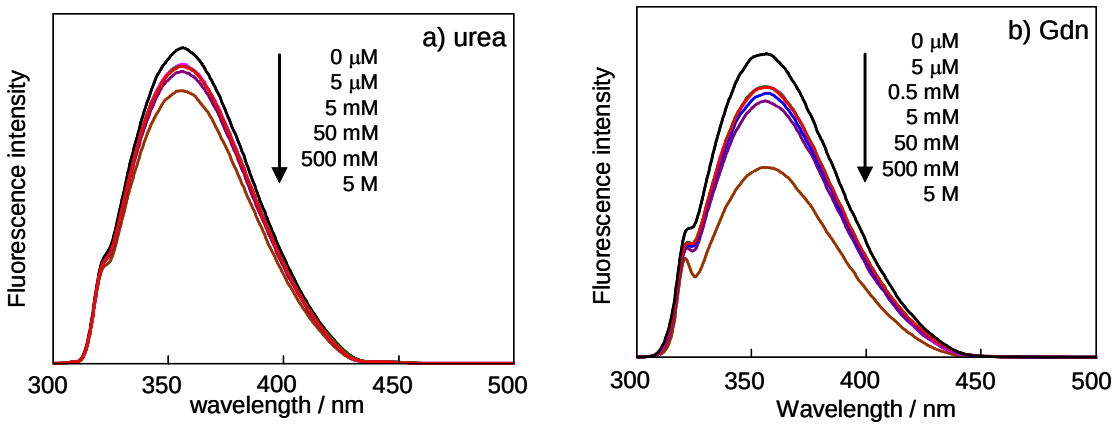
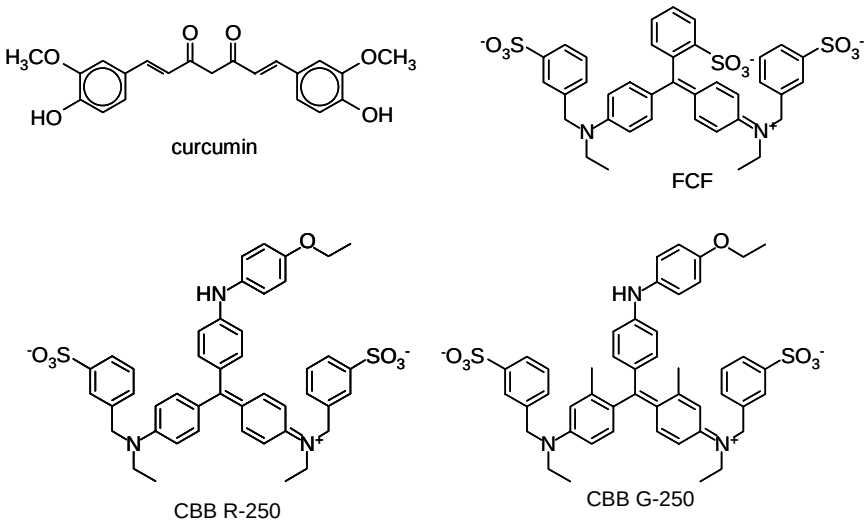


Fig. S5 Fluorescence spectra of Trp (1 μM) with a) urea and b) Gdn in 10 mM phosphate buffer (pH 7.0) at 25 °C. λ_{ex} = 290 nm.

Chemical structures of curcumin and blue dyes used in this study.



Scheme 1S Chemical structures of various organic molecules.

ThT fluorescence assay of Aβ(1-40) HCl salt with BB-FCF.

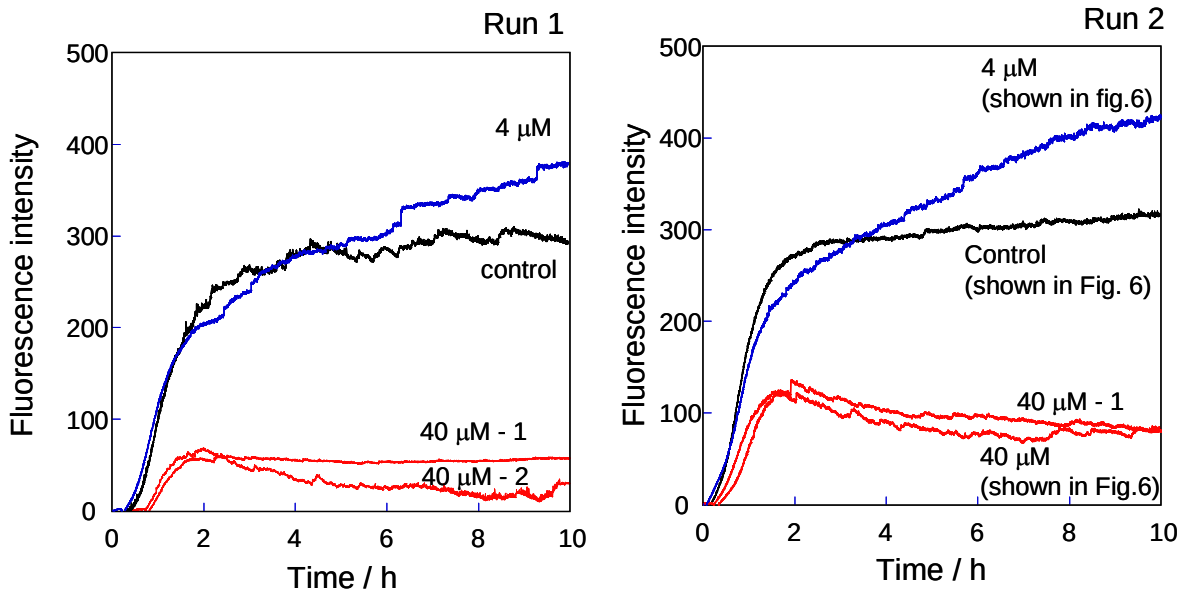


Fig. 6S Temporal changes of ThT fluorescence intensity at 480 nm (λ_{ex} = 442 nm) with BB-FCF in 50 mM phosphate buffer (pH 7.4) at 37°C. C. [Aβ(1-40)] = 5 μM.